

In the Claims

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing.

Please amend pending claims 30, 33 and 46 as indicated below.

Please add new claims 58-60 as provided below.

1.-29. (Cancelled)

30. (Currently Amended) A method of specifically cleaving a heparin-like glycosaminoglycan, comprising

contacting a heparin-like glycosaminoglycan with the heparinase of any one of:

a substantially pure heparinase comprising a modified heparinase II, and

a substantially pure heparinase comprising a modified heparinase I,

wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 or having conservative substitutions thereof, wherein at least one amino acid residue is substituted and the substitution is selected from the group consisting of (a) a cysteine residue corresponding to position 348 substituted with a different amino acid than in native heparinase II; (b) a histidine residue corresponding to at least one of positions 238, 252, 347, 440, 451, and 579 substituted with alanine, serine, tyrosine, threonine, or lysine; and (c) a conservative substitution of a heparin-binding sequence residue corresponding to at least one of positions 446-451, ~~is substituted with a different amino acid than in native heparinase II,~~

and wherein the modified heparinase I has the amino acid sequence of the mature peptide of SEQ ID NO: 4 or having conservative substitutions thereof, wherein at least one amino acid residue ~~is has been substituted and the substitution is~~ with a different amino acid than in native heparinase I, and wherein the residue that has been substituted is a serine residue corresponding to position 377 substituted with alanine, serine, tyrosine, histidine, threonine, or lysine.

31. (Original) The method of claim 30, wherein the heparin-like glycosaminoglycan is contacted with a modified heparinase II, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein the histidine residue corresponding to

position 440 of SEQ. ID NO: 2 is substituted with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine to specifically cleave a heparin-like glycosaminoglycan.

32. (Original) The method of claim 30, wherein the heparin-like glycosaminoglycan is contacted with a modified heparinase I, wherein the modified heparinase I has the amino acid sequence of the mature peptide of SEQ ID NO: 4 wherein at least one amino acid residue has been substituted and wherein the substitution is a substitution of a serine residue corresponding to position 377 of SEQ ID NO: 4 with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine.

33. (Currently Amended) The method of claim[[s]] 30, wherein the method is a method of removing heparin from a heparin containing fluid.

34. (Original) The method of claim 33, wherein the heparinase is immobilized on a solid support.

35.-45. (Canceled)

46. (Currently Amended) A method of specifically cleaving a heparan sulfate-like glycosaminoglycan

comprising contacting a heparan sulfate containing fluid with a substantially pure heparinase comprising a modified heparinase II,

wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 or having conservative substitutions thereof, wherein at least one amino acid residue is substituted and the substitution is selected from the group consisting of (a) a cysteine residue corresponding to position 348 substituted with a different amino acid than in native heparinase II; (b) a histidine residue corresponding to at least one of positions 238, 252, 347, 440, 451, and 579 substituted with alanine, serine, tyrosine, threonine, or lysine; and (c) a

conservative substitution of a heparin-binding sequence residue corresponding to at least one of positions 446-451, ~~is substituted with a different amino acid than in native heparinase II.~~

47. (Previously Presented) The method of claim 46, wherein the method is a method of removing heparan sulfate from a heparan sulfate containing fluid.

48. (Original) The method of claim 47 wherein the heparinase is immobilized on a solid support.

49. (Original) The method of claim 46, wherein the heparan sulfate-like glycosaminoglycan is contacted with a substantially pure modified heparinase II, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein the cysteine residue corresponding to position 348 of SEQ ID NO: 2 has been substituted with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine to specifically cleave a heparin sulfate-like glycosaminoglycan.

50.-57. (Canceled)

58. (New) The method of claim 30, wherein the at least one substituted residue of the modified heparinase II is the cysteine residue corresponding to position 348 substituted with alanine.

59. (New) The method of claim 30, wherein the at least one substituted residue of the modified heparinase I is the serine residue corresponding to position 377 substituted with alanine.

60. (New) The method of claim 46, wherein the at least one substituted residue of the modified heparinase II is the cysteine residue corresponding to position 348 substituted with alanine.